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# A Genome-Wide Study on the Perception of the Odorants Androstenone and Galaxolide

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## Abstract

Twin pairs and their siblings rated the intensity of the odorants amyl acetate, androstenone, eugenol, Galaxolide, mercaptans, and rose ($N = 1573$). Heritability was established for ratings of androstenone ($h^2 = 0.30$) and Galaxolide ($h^2 = 0.34$) but not for the other odorants. Genome-wide association analysis using 2.3 million single nucleotide polymorphisms indicated that the most significant association was between androstenone and a region without known olfactory receptor genes (*rs10966900*, $P = 1.2 \times 10^{-7}$). A previously reported association between the olfactory receptor *OR7D4* and the androstenone was not detected until we specifically typed this gene ($P = 1.1 \times 10^{-4}$). We also tested these 2 associations in a second independent sample of subjects and replicated the results either fully (*OR7D4*, $P = 0.00002$) or partially (*rs10966900*, $P = 0.010$; $N = 266$). These findings suggest that 1) the perceived intensity of some but not all odorants is a heritable trait, 2) use of a current genome-wide marker panel did not detect a known olfactory genotype–phenotype association, and 3) person-to-person differences in androstenone perception are influenced by *OR7D4* genotype and perhaps by variants of other genes.

**Key words:** androstenone, Galaxolide, genetic twin modeling, genome-wide association study, heritability, twins

## Introduction

Some individuals with an otherwise normal sense of smell are unable to detect the odor of androstenone (5α-androst-16-en-3-one) at the concentrations tested, and those who are able to perceive it describe the odor in different ways: as sweaty, urinous, musky, sweet, or even perfume-like (Griffiths and Patterson 1970; Gilbert and Wysocki 1987). Like androstenone, Galaxolide, a musky odorant, cannot be detected by some individuals (Wysocki and Gilbert 1989; Baydar et al. 1993). Galaxolide differs from androstenone in that most people who can smell it find it pleasant (Wysocki and Gilbert 1989). The term ''specific allosmia'' describes the diversity of quality descriptors for a given odorant (O'Connell et al. 1994), and the term specific anosmia describes the inability of some people to smell an odorant (Amoore 1967). Therefore, the perception of androstenone is an example of both a specific allosmia and anosmia, whereas the perception of Galaxolide is a specific anosmia.

The ability to detect androstenone is a heritable trait; that is, genetic variation accounts for a significant proportion of person-to-person differences (Wysocki and Beauchamp 1984; Gross-Isseroff et al. 1992; Pause et al. 1998; Keller et al. 2007; Knaapila, Tuorila, Silventoinen, Wright, Kyvik, Cherkas, et al. 2008). Heritability for the sensitivity to pentadecalactone, another musky odorant, also has been demonstrated (Whissell-Buechy and Amoore 1973). However, the heritability for the perception of other odorants, including Galaxolide, has not been established. Although it might be tempting to assume that any differences extreme enough to be considered a specific anosmia would be heritable, this is not a tenable assumption. Even when there is a wide range of perceptual abilities in the population for a given odorant, the ability to smell it is often not a heritable trait (Hubert et al. 1980; Forrai et al. 1981; Knaapila, Tuorila, Silventoinen, Wright, Kyvik, Keskitalo, et al. 2008). Therefore, the relative

contribution of genes and environment to olfactory thresholds must be evaluated for each odorant.

Geneticists have been interested in the ability to smell androstenone because the expectation is that individual differences can be explained by a deleterious allele in a particular narrowly tuned olfactory receptor (Guillot 1948; Amoore 1967). This hypothesis has proved to be partially true: alleles in the olfactory receptor gene *OR7D4* explained 39% of the variance in the intensity ratings of androstenone (Keller et al. 2007). Associations between olfactory receptor alleles and perception are observed not only for androstenone and androstadienone (Keller et al. 2007) but also for isovaleric acid (Menashe et al. 2007), asparagus metabolites (Eriksson et al. 2010; Pelchat et al. 2010), and *cis*-3-hexen-1-ol (Jaeger et al. 2010). However, these genetic associations explain only a fraction of the variation in olfactory ability among people. Therefore, we wondered whether alleles in multiple olfactory receptors or alleles in other types of genes might contribute to individual differences in smell. To examine this question, we undertook a large-scale association study in which we assessed the heritability of the perception of several pure odorants and odorant mixtures in twin families. For those odorants for which heritability was detected, we applied a hypothesis-free approach, the genome-wide association study, to discover genetic variants associated with their perception. This method searches for associations between a trait and a large number of polymorphisms (~250 K to >2 M) selected to densely cover the entire genome (Manolio 2010). To confirm potential associations indicated by the genome-wide association study, we tested the results in a second independent population sample.

## Materials and methods

### General approach

We measured ratings of odorant intensity using the National Geographic Smell Survey (NGSS) and University of Helsinki Smell Survey (UHSS), and heritability estimates were calculated for each of the odorant traits. Those with evidence of heritability were screened for associations with the 2.3 million single nucleotide polymorphism (SNP) markers. The genetic variants most strongly associated with odorant perception in the genome-wide association analysis were genotyped in an independent sample of similar ancestry. Subjects genotyped for the genome-wide association study are referred to as the discovery sample, and those genotyped to confirm these results are called the replication sample.

### Participants

#### *Discovery sample*

Participants were Australian (Caucasian) adolescent and young-adult twins and their singleton siblings, 10–25 years of age (mean, 18 ± 3 years), from the Brisbane adolescent twin study ($N$ = 1573, including 872 females and 701 males) (Wright and Martin 2004). The study protocols were approved by the Queensland Institute of Medical Research Human Research Ethics Committee, and participants (and their parents for participants <18 years of age) gave informed consent before inclusion in the study.

Zygosity of same-sex twins was established by DNA typing of 9 markers (AmpF1STR Profiler Plus Amplification KIT, Applied Biosystems Inc.) as described previously (Hansen et al. 2006) and later confirmed by genotyping from the 610K SNP chip (see Genotyping). Heritability results from a subset of the participants (~200 individuals) have been reported previously (Knaapila, Tuorila, Silventoinen, Wright, Kyvik, Cherkas, et al. 2008; Knaapila, Tuorila, Silventoinen, Wright, Kyvik, Keskitalo, et al. 2008).

#### *Replication sample*

Participants were American (Caucasian) adult twins 21–80 years of age (mean, 38 ± 16 years) who took part in a chemosensory study at the annual Twins Days festival (Twinsburg, Ohio) and provided valid test responses ($N$ = 226, including 180 women and 46 men, 100 monozygous [MZ] and 13 dizygous [DZ] twin pairs). Zygosity was determined by self-report, physical appearance, and the results of genotyping 40 markers, with no discrepancies among the reported and observed zygosity. The replication study was performed with the approval of the Institutional Review Board at the University of Pennsylvania, and informed consent was obtained from all participants.

### Odor stimuli and rating scales

#### *Discovery sample*

Each participant completed 1 of 2 smell surveys: the NGSS ($N$ = 992, including 556 females and 436 males, 11–25 years of age, mean 18 ± 3 years) (Wysocki and Gilbert 1989) or the UHSS ($N$ = 594, including 327 females and 267 males, 10–20 years of age, mean 14 ± 2 years) (Knaapila, Tuorila, Silventoinen, Wright, Kyvik, Cherkas, et al. 2008; Knaapila, Tuorila, Silventoinen, Wright, Kyvik, Keskitalo, et al. 2008); 13 participants (11 females and 2 males) completed both surveys, for a total of 1583 completed surveys. The NGSS was mailed to the participants, who completed the test at home and returned it to the research unit by mail. The UHSS was taken under supervision in the clinic at the Queensland Institute of Medical Research. Both stimuli sets contained odorants microencapsulated into separate scratch-and-sniff panels that are released by scratching the panels using a pencil or coin, followed by sniffing and evaluating the released odorants. Six stimuli were included in the NGSS (androstenone, Galaxolide, eugenol, isoamyl acetate, mercaptans, and synthetic rose), and 6 stimuli were included in the UHSS (androstenone, chocolate, cinnamon, isovaleric acid, lemon, and turpentine). Androstenone was included in both tests.

The NGSS was used to measure responses to detection, pleasantness, and perceived intensity of the odor stimuli.

Participants first answered "Yes" or "No" to the question "Did you smell something?" If the answer was "Yes," the participant rated the odor's pleasantness ("How would you rate the quality of this odor"?) and perceived intensity ("How intense is this odor"?) on a scale from 1 to 5, with endpoints anchored as "Unpleasant" (1) and "Pleasant" (5) in the pleasantness scale and as "Weak" (1) and "Strong" (5) in the intensity scale. For the NGSS, we assigned an intensity rating of 0 to those participants unable to detect the odor, so the NGSS range of values included 6 categories, ranging from 0 for "No odor" to 5 for "Strong."

The UHSS was used to measure responses to pleasantness and perceived intensity. The participants were asked to rate each odor's pleasantness ("Rate the pleasantness of the odor") and perceived intensity ("Rate the intensity of the odor") on scales from 1 to 9, with endpoints anchored as "Extremely unpleasant" (1) and "Extremely pleasant" (9) in the pleasantness scale and as "No odor" (1) and "Extremely strong odor" (9) in the intensity scale.

### *Replication sample*

In the replication sample, the phenotyping method was changed for practical reasons (the NGSS and UHSS surveys were no longer available). Instead, participants smelled solutions of androstenone (Sigma A8008; 0.05% wt/vol in mineral oil) and Galaxolide (International Flavors & Fragrances Inc.; 5% wt/wt in mineral oil) in a series with other taste and smell stimuli (these results are not reported here). The CAS numbers and IUPAC names are reported in the Electronic and Chemical Resources section, below. Similar to the discovery sample NGSS testing, participants first answered "Yes" or "No" to the question "Did you smell something"? If the answer was "Yes," the participant rated the odor's liking ("How much do you like the odor"?) and perceived intensity ("How intense is the odor"?) on a 7.8-cm visual analogue scale. The scale for rating the liking was anchored with "Do not like at all" (left), "Neutral" (middle), and "Like extremely" (right). The scale for rating the intensity was anchored with "Like air" (left), "Moderate" (middle), and "Strongest imaginable" (right). Similar to the analysis of the data from the discovery sample described above, we assigned an intensity rating of 0 to those participants unable to detect the odor. For nonzero values, we measured the distance marked by the line, that is, up to 7.8 cm. Twenty subjects were retested, and we observed significant test–retest reliability for the intensity ratings of both androstenone (Pearson $r = 0.65$, $P = 0.0007$) and Galaxolide (Pearson $r = 0.70$, $P = 0.00017$).

## Genotyping

### *Discovery sample*

DNA was extracted from blood, and genotyping was performed with the Illumina 610-Quad BeadChip system (Illumina Inc.). A total of 529 721 SNPs passed quality control, as described previously (Medland et al. 2009). To gain the maximum amount of potential information for the association study, genomic coverage was extended to 2.3 million SNPs by imputation using the phased data from the HapMap samples of Caucasian European ancestry (CEU, Build 36, Release 22) and MACH 1.0 Markov chain–based haplotyper (Li and Abecasis 2006). Quality control filters were applied to the assayed genotypes to restrict the imputation to samples and SNPs with high data quality (i.e., imputation score <0.3 [indicating low imputation confidence; ~3%], a minor allele frequency <0.01, or a Hardy–Weinberg equilibrium score of $P < 10^{-6}$ [~5%]). To specifically test for an association with *OR7D4*, an olfactory receptor associated with androstenone sensitivity, DNA was typed in all samples by a commercial service (KBioscience) for the functional variant *rs61729907* (*OR7D4* R88W).

### *Replication sample*

DNA was extracted from saliva (DNA Genotek) and genotyped with the Applied Biosciences TaqMan genotyping assay using ABI Real-Time PCR (Life Technologies Corporation) as described previously (Mennella et al. 2005). Three markers were genotyped: *rs10966900*, *rs61729907*, and *rs3819256*.

## Data analysis

### *Preliminary analysis*

The phenotype data were examined for logical inconsistencies, and 2 participants were excluded from the NGSS sample and 2 from the UHSS for invalid responses. Using the statistical package PASW Statistics 17 (SPSS Inc.), we standardized the scores for the 2 different surveys by calculating normal weighted scores (expressed relative to the mean [set to zero] and the standard deviation (SD) so they can be compared and pooled) and corrected them for age and sex. We then used the standardized residuals for all further analyses of the discovery sample phenotype data. To maximize sample size for the genetic analyses, we pooled the standardized scores for androstenone from the NGSS and UHSS; for the 13 participants who completed both smell surveys, we used the NGSS scores. This procedure gave a final combined sample size of 1569 participants (173 MZ and 394 DZ pairs; 108 single twins, 327 nontwin siblings).

### *Heritability: correlations and modeling*

We used the statistical package Mx (Neale et al. 2002) to calculate maximum likelihood twin correlations and used univariate structural equation modeling to estimate the sources of variance. Using the twin design, the phenotypic variance of the responses to odors can be decomposed into additive genetic effects (A), shared (common) environmental effects (C), and nonshared (individual) environmental effects (E).

The significance of the variance components was tested by comparing $\chi^2$ statistics (measuring the fit of the model to the data) of the nested models. The fit of the submodels (AE/CE/E models) was tested against the full ACE model. If the fit of the model without the A component is significantly worse than the model including it (i.e., has a significantly larger $-2 \times$ log likelihood value after taking into account the decrease of the degrees of freedom [dfs]), then the A component (and corresponding heritability estimate) is regarded as a reliable estimate.

### *Genome-wide association*

Associations between heritable traits and 2.3 million SNPs were explored to identify underlying genetic variants. Individual SNPs were tested for association with the family-based SCORE test implemented in the software program Merlin (Chen and Abecasis 2007), which accounts for the relatedness of individuals, including MZ twins.

### *Genotype association for the replication sample*

Subjects were grouped by genotype, and the average ratings of perceived intensity were compared using Kruskal–Wallis one-way analysis of variance. This nonparametric statistical test was selected because ratings of intensity were not normally distributed.

## Results

### Odor detection

The majority of participants provided valid detection and intensity responses for all 6 odors in the NGSS (985 of 992 [99.3%]) or UHSS (581 of 594 [97.8%]) and were able to smell most odorants (all 6 odors detected by 60.4% [NGSS] and 83.6% [UHSS] of the participants), with very few potential general anosmic individuals (only 0.2% of the NGSS and UHSS samples were unable to detect any of the odorants). Androstenone was not detected by 17.6% (NGSS) and 10.8% (UHSS) of participants, Galaxolide by 11.7% (Figure 1), and mercaptans by 19.5%. All other odor stimuli went undetected by <1% (NGSS) and <5% (UHSS) of the participants.

Females rated Galaxolide as more intense than did males (Mann–Whitney *U*-test, $Z = -2.99$, $P = 0.003$); gender differences were not significant for intensity ratings of androstenone in the NGSS ($Z = -1.68$, $P = 0.09$) or in the UHSS ($Z = -0.21$, $P = 0.83$). Age was negatively correlated with intensity ratings of androstenone in both the NGSS (Spearman's $\rho = -0.16$, $P < 0.001$) and UHSS ($\rho = -0.13$, $P = 0.002$). In contrast, no correlation was observed between age and intensity ratings of Galaxolide ($\rho = -0.08$, $P = 0.80$). The phenotypic correlation between androstenone and Galaxolide sensitivity was $r = 0.17$ ($P < 0.01$; $N = 977$).

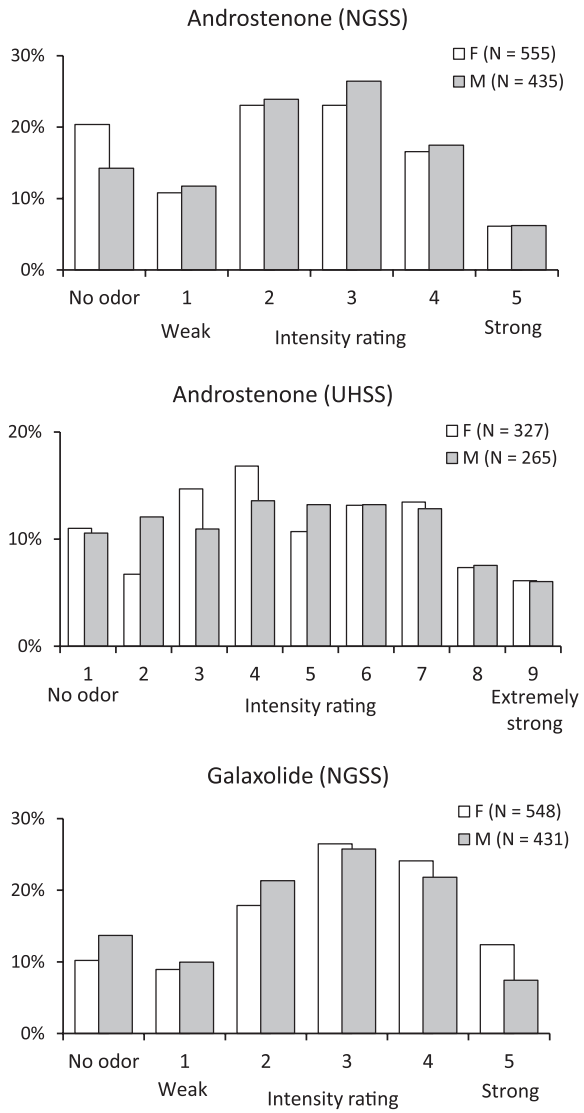


**Figure 1** Frequencies of responses to perceived intensity of (upper panel) androstenone in NGSS (National Geographic Smell Survey; valid response for 990 of the 992 participants), (middle panel) androstenone in UHSS (University of Helsinki Smell Survey; valid response for 592 of 594), and (lower panel) Galaxolide in NGSS (valid response for 979 of 992). F, females; M, males.

### Heritability analysis

Heritability was significant for the intensity ratings of androstenone and Galaxolide but not for the other odorants. For both androstenone and Galaxolide, the MZ twin correlation ($r_{MZ}$) was significant (the lower limit of the 95% confidence interval was greater than zero) and higher than that of the DZ correlation ($r_{DZ}$), suggesting a genetic influence. ACE genetic modeling estimates for heritability (additive genetic effects) were significant and moderate for the intensity ratings of androstenone (0.28–0.30) and Galaxolide (0.34; Table 1). There was no evidence of a genetic influence for intensity ratings of the other odorants. There was also little

evidence of a genetic influence for the pleasantness ratings (see twin correlations in Supplementary Table 1). Therefore, these data were excluded from further genotype–phenotype association analyses.

### Genome-wide association

Genome-wide associations for the intensity ratings of androstenone are depicted in Figure 2 and for Galaxolide in Figure 3. None of the markers reached a genome-wide statistical criterion of $1.14 \times 10^{-8}$ (Medland et al. 2009), a level that corrects for the 1 million independent common variants in the genome (Anonymous 2003). For androstenone, the strongest association was observed on chromosome 9p21.3 (*rs10966900*, $P = 1.2 \times 10^{-7}$; accounting for 2.4% of the variance). For Galaxolide, the most significant associations clustered on chromosome 11q14.1; the top hit (*rs3819256*, $P = 1.1 \times 10^{-6}$; accounting for 3.8% of the variance) was located within the gene *INTS4* (integrator complex subunit 4). A search was undertaken within 20 MB of these markers for olfactory receptors including those which were pseudogenes, but none were found. The most highly associated SNPs for androstenone and Galaxolide are listed in Table 2. Ratios of expected and observed *P* values for intensity ratings of androstenone and Galaxolide are depicted as Q–Q plots in Figure 4. The genomic inflation factor (lambda) ranged between 0.995 and 1.014, indicating that potential technical or population stratification artifacts had a negligible impact on the results.

We also examined the previous relationship with *OR7D4*, a gene associated with androstenone perception (Keller et al. 2007); although 2 genotyped markers flanked this gene (*rs10407714* and *rs8101575*), neither was related to ratings of androstenone intensity ($P = 0.25$ and $P = 0.79$, respectively). Likewise, none of the 108 markers within a 100 kb window of the *OR7D4* gene were related ($P > 0.05$). The physical distance between the 2 markers closest to the *OR7D4* gene is 9.4 kb (GRCh Build 37), but the linkage disequilibrium between them is low ($r^2 = 0.16$; HapMap data, release #18; see Electronic Resources). We therefore genotyped a causal genetic variant within the *OR7D4* gene (R88W; *rs61729907*) and demonstrated that this variant was associated with androstenone ratings ($P = 0.001$; Figure 5). We also used this *OR7D4* genotype data to evaluate linkage disequilibrium with the existing marker coverage in this region and found that the $r^2$ was generally low (average $r^2 < 0.06$, SD = 0.12), with maximum linkage disequilibrium ($r^2$) of 0.65 between *rs61729907* (*OR7D4*) and a marker approximately 20 kb away, near a related olfactory receptor gene, *OR7D2* (*rs878246*).

### Replication sample

Androstenone was not detected by 48.7% and Galaxolide by 23.5% of the people in the replication sample. There were no sex differences in intensity ratings for Galaxolide or androstenone (Mann–Whitney *U*-test, $Z = -0.74$, $P = 0.46$; $Z = -1.19$, $P = 0.24$). Age was correlated with the intensity ratings of androstenone (Spearman's $\rho = 0.14$, $P = 0.04$) but not Galaxolide ($\rho = 0.08$, $P = 0.22$). There was a correlation between androstenone and Galaxolide sensitivity ($\rho = 0.27$, $P < 0.001$) that was higher than that observed in the discovery sample.

In this sample, we again replicated the previously reported association between perception of androstenone and *OR7D4* (Keller et al. 2007; *rs61729907*, $\chi^2 = 21.96$, df = 2, $P = 1.71 \times 10^{-5}$; Figure 6). For Galaxolide, we tested but did not replicate the association between its perception and alleles of *rs3819256* ($\chi^2 = 1.02$, df = 2, $P = 0.60$). We tested the relationship between perception of androstenone and alleles of *rs10966900* and partially confirmed the association found in the discovery sample ($\chi^2 = 9.22$, df = 2, $P = 0.010$; Figure 6). We say partially because the direction of the allelic effects differed between the samples, with 2 copies of the minor allele being markedly associated with increased sensitivity in the replication sample but decreased sensitivity in the discovery sample.

**Table 1** Twin correlations and results from heritability analysis for intensity ratings of the odors of androstenone and Galaxolide

| Odorant and sample | *N* complete twin pairs (MZ/DZ) | Twin correlations (95% CI) | Parameter estimates of the variance components (95% CI) | | | Significance of the additive genetic effects | |
|---|---|---|---|---|---|---|---|
| | | $r_{MZ}$ | $r_{DZ}$ | $a^2$ | $e^2$ | Δ-2LL | *P* value |
| Androstenone | | | | | | | |
| NGSS | 110/228 | 0.37 (0.21, 0.50) | 0.02 (0.00, 0.15) | 0.28 (0.14, 0.42) | 0.72 (0.58, 0.86) | 14.206 | 0.001 |
| UHSS | 65/170 | 0.33 (0.09, 0.51) | 0.17 (0.02, 0.30) | 0.33 (0.15, 0.49) | 0.66 (0.54, 0.80) | 11.876 | 0.003 |
| Combined | 173/394 | 0.37 (0.24, 0.48) | 0.08 (0.00, 0.18) | 0.30 (0.19, 0.41) | 0.70 (0.59, 0.81) | 25.747 | <0.001 |
| Galaxolide | | | | | | | |
| NGSS | 109/220 | 0.38 (0.22, 0.50) | 0.10 (0.00, 0.23) | 0.34 (0.20, 0.46) | 0.66 (0.54, 0.80) | 20.872 | <0.001 |

CI, confidence interval; $a^2$, additive genetic effects (heritability); $e^2$, nonshared environmental effects; Δ-2LL, $-2 \times$ log likelihood.

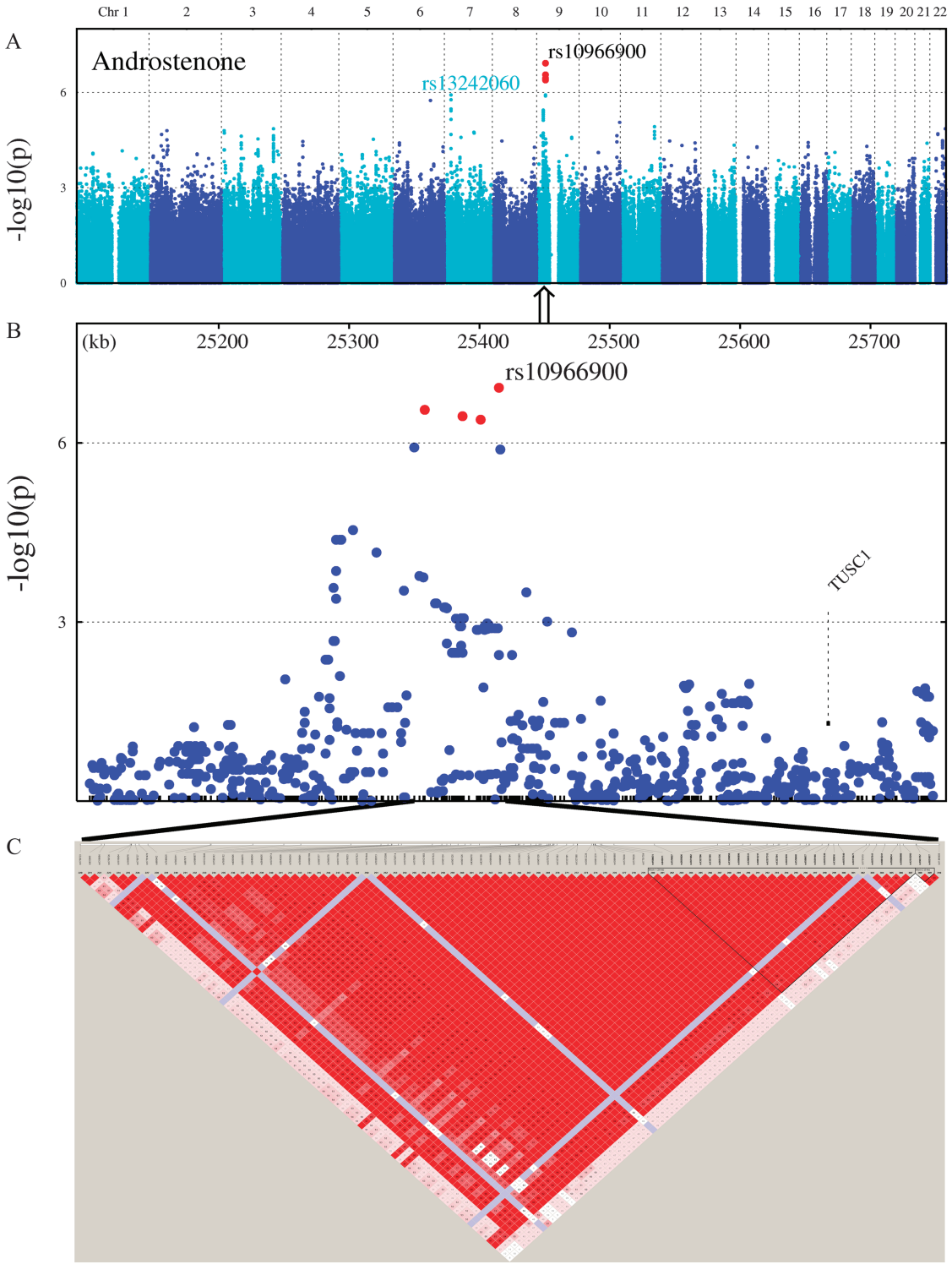


**Figure 2** Manhattan plot showing genome-wide association for the intensity rating of androstenone (panel A), followed by regional association between 9p21.3 variants and androstenone perception (panel B), and linkage disequilibrium (heat map) among markers for the 9p21.3 region (panel C). In panel A, results are plotted as negative log-transformed *P* values from the genotypic association test (observed −log 10 *P* values by position [Mbp]); the horizontal dotted gray line indicates $P = 10^{-6}$. Odd chromosome numbers are in light blue, and even chromosome numbers in dark blue. One genomic region (9p21.3) contained 4 SNPs that exceeded the genome-wide level of $10^{-6}$ (red dots). In panel B, the regional association plot between 9p21.3 variants and androstenone perception indicates the location of the only known gene (TUSC1). SNPs with a *P* value $10^{-6}$ are shown in red.

## Discussion

### Genetics contributed to perception of some but not all odorants

We explored the genetic contribution to perceived intensity and pleasantness of Galaxolide and androstenone, 2 odorants that are characterized as musky by some people and of other nonmusk odorants (e.g., isoamyl acetate and eugenol). For androstenone, about one-third of the variance in perceived intensity could be explained by additive genetic factors, which matches previous reports (Wysocki and Beauchamp 1984; Gross-Isseroff et al. 1992; Knaapila, Tuorila, Silventoinen, Wright, Kyvik, Cherkas, et al. 2008). In addition, we found that the heritability of perceived Galaxolide intensity was similar to that of androstenone. But these were the only odorants of those tested for which we detected genetic effects for perceived intensity.

These observations support the notion that genetic factors contribute to the perception of musky odorants, a hypothesis consistent with the results of a family segregation study of the perception of the musky odorant pentadecalactone (Whissell-Buechy and Amoore 1973). Our data also support previous observations of the lack of genetic effects on the perceived intensity for nonmusk odorants (Knaapila, Tuorila, Silventoinen, Wright, Kyvik, Keskitalo, et al. 2008), although, in general, studies about odor heritability have been mixed. For instance, the hyperosmia to isovaleric acid (Menashe et al. 2007) and the detection of isoamyl acetate (Gross-Isseroff et al. 1992) are heritable, but sensitivity to acetic acid, isobutyric acid, or 2-sec-butyl-cyclohexanone is not (Hubert et al. 1980). Although we conclude from the current data that the perception is more heritable for certain types of odorants than for others, this is true only for perception assessed by the methods employed here (scratch and sniff). It is possible that more sensitive measures of olfactory function could uncover heritability of perceived intensity for most or all odorants. We observed no heritability for perceived pleasantness of any odorant tested. This observation is consistent with the hypothesis that odor pleasantness is largely determined by experience and learning that is specific to an individual (Hudson 1999).

### The hypothesis that SNPs in a genome-wide association panel would link to odorant receptors was not supported

Our expectation was that the genome-wide association study would identify variants within regions of the genome that contain clusters of olfactory receptor genes and that alleles of one or more of those genes would account for some of the variation in the perception of androstenone or Galaxolide. This was not the case. Instead, we found one association in an intergenic interval between *TUSC1* and *ELAVL2*, and we found the previously reported association with the olfactory receptor *OR7D4* but only after we specifically genotyped that particular gene. Our current results agree with those of Pollack et al. (1982) in not finding a relationship between sensitivity to the odor of androstenone and alleles of the Human leukocyte antigen region (Pollack et al. 1982).

One reason that the genome-wide association analysis did not detect associations within or near olfactory receptors is because these genes may not be adequately represented on the high-throughput genotyping panels. Olfactory receptors have similar nucleotide sequences and are especially likely to be in regions with dense copy number variation (Hasin et al. 2008), which may make them poor targets for genetic marker development. For example, we observed through database searches of Ensembl (see Electronic Resources) that the genotyping platform used herein (Illumina Human 660W Quad) has markers within only 38% of the olfactory receptor genes. Although lack of olfactory receptor coverage may explain our genome-wide association results, the extent to which the lack of olfactory receptor markers might have obscured detection of these associations is not possible to judge without genotyping olfactory receptor polymorphisms.

### Genetic variation on chromosome 9 may contribute to androstenone perception

We found a suggestive but not significant association for androstenone perception in a region of the genome without known olfactory receptors, between *ELAVL2* and *TUSC1* on chromosome 9. Little is known about the *TUSC1* gene except that it is deleted in some forms of lung cancer and is thus named Tumor Suppressor Candidate 1. It is widely expressed in many human tissues, such as kidney, heart, and brain (Shan et al. 2004). Screens of rodent olfactory receptor neuron cilia have not reported the *TUSC1* gene or its protein product (Mayer et al. 2008; McClintock et al. 2008; Mayer et al. 2009; Stephan et al. 2009). The *ELAVL2* gene may be a better candidate gene because it is involved in neural development (Akamatsu et al. 1999), and its expression changes when mice learn to make odor discriminations (Smalheiser et al. 2010). The *ELAVL2* gene may be involved in the induction of androstenone sensitivity with experience (Wysocki et al. 1989). The direction of the allelic effect differed between the discovery sample and the replication sample, which may indicate that the association was spurious, or it may be an instance of a true association, and the allelic "flip-flop" is due to differences in the underlying population structure between U.S. and Australian Caucasians (Lin et al. 2007). It is more likely that these results are due to an age-dependent genotype effect. The subjects in the discovery sample were adolescents, whereas the average age of the replication sample was >40 years. Although the heterogeneity of age is a limitation of this study, it may be that this gene is involved in olfactory learning, and its effects may be age dependent. Additional studies will be needed to resolve this issue.

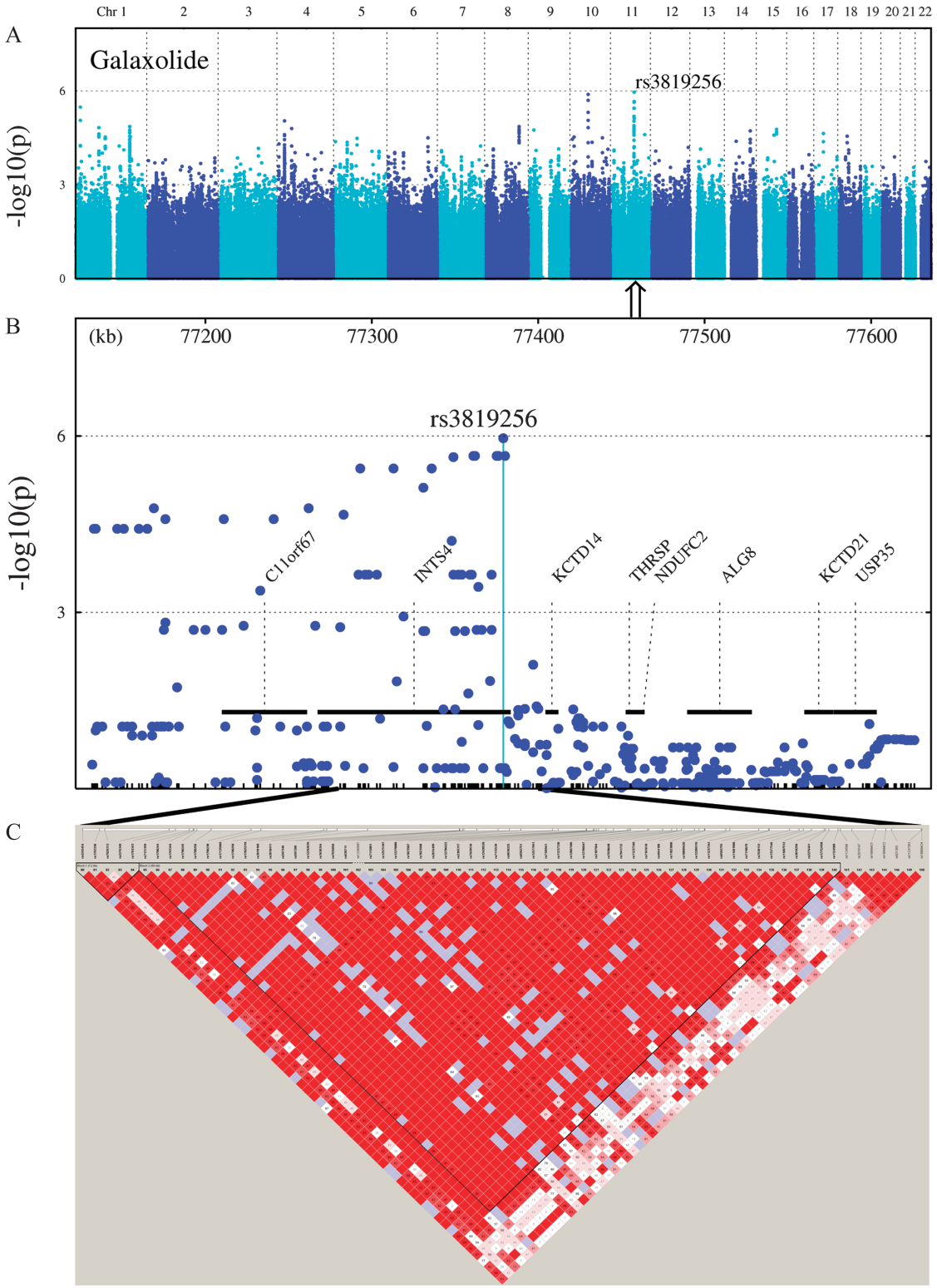


**Figure 3** Manhattan plot showing genome-wide association for the intensity rating of Galaxolide (panel A), followed by regional association between 11q14.1 variants and Galaxolide perception (panel B), and linkage disequilibrium (heat map) among markers for the 11q14.1 region (panel C). See Figure 2 for details.

From a genome-wide perspective, the association results suggest that androstenone and Galaxolide have no genomic regions in common, indicating that the genetic variants that lead to these perceptual differences arise through different mechanisms. Although the sample size may be too small to draw this conclusion unequivocally, this conclusion is also

**Table 2** Top SNPs associated with intensity ratings of androstenone and Galaxolide

| Odorant | SNP[a] | Chr | Location | MAF | *P* value | $h^2$ (%) | Gene[b] |
|---|---|---|---|---|---|---|---|
| Androstenone | *rs10966900* | 9 | 25415160 | 0.16 | $1.2 \times 10^{-07}$ | 2.4 | *ELAVL2*[c] |
| | *rs13231337* | 7 | 17526355 | 0.12 | $1.2 \times 10^{-06}$ | 2.0 | *AHR* |
| Galaxolide | *rs3819256* | 11 | 77379509 | 0.50 | $1.1 \times 10^{-06}$ | 3.8 | *INTS4* |
| | *rs12414237* | 10 | 58367222 | 0.11 | $1.3 \times 10^{-06}$ | 3.4 | *ZWINT* |

Chr = Chromosome number; MAF = minor allele frequency; $h^2$ % = percentage of additive genetic variance.
[a]Other associated markers in high linkage disequilibrium include the following: *rs10966900—rs10122848*, *rs17701704*, *rs1327395*, *rs7862611*, and *rs7848925*; *rs13231337—rs13242060*, *rs13231096*, and *rs13228338*; *rs3819256—rs2276441*, *rs11237340*, and *rs2034499*; *rs12414237—rs16909785*, *rs7938648*, *rs7938133*, and *rs7110670*.
[b]All marker locations are from GRCh Build 37. Where the SNP is not located within a gene, the closest gene is indicated.
[c]Marker is between the *TUSC1* and the *ELAVL2* genes.

supported by the weak phenotypic correlations between the perception of androstenone and Galaxolide in both the discovery and the replication samples. In addition, the androstenone association results are in partial agreement with another genome-wide association study that examined the detection of *cis*-3-hexen-1-ol (an odorant found in fruits and vegetables) (Jaeger et al. 2010). The most strongly associated regions from that study included a SNP (*rs16932288*, Chr 9, position 14781444) within about 10 Mb of the regions identified here for androstenone (*rs10966900*, Chr 9, position 25425160). The SNPs are unlikely to be in linkage disequilibrium, but clustered genes with similar functions may account for this pattern of results between these studies.

### Results suggest a rethinking the genetics of specific anosmias

We began this work assuming that specific anosmias were single-gene traits, accounted for by loss of an olfactory receptor tuned to a particular odorant. But our results only partially support this hypothesis. In fact, several associations were detected in the discovery sample, and all were in regions of the genome without known olfactory receptors. Although we could detect an association between androstenone perception and its previously identified receptor *OR7D4* in all populations tested, this gene did not fully account for genetic variance among people, in agreement with results of Keller et al. (2007). The results also did not support a polygenic model with multiple olfactory genes (Krautwurst et al. 1998; Malnic et al. 1999), although we cannot draw a firm conclusion on this point because olfactory receptors could be missed due to low marker coverage. The interpretation of our data we favor is that, in addition to olfactory receptor genes, one or more other polymorphic genes are associated with the inability to smell androstenone and Galaxolide. This inability may be due to genes or regulatory elements

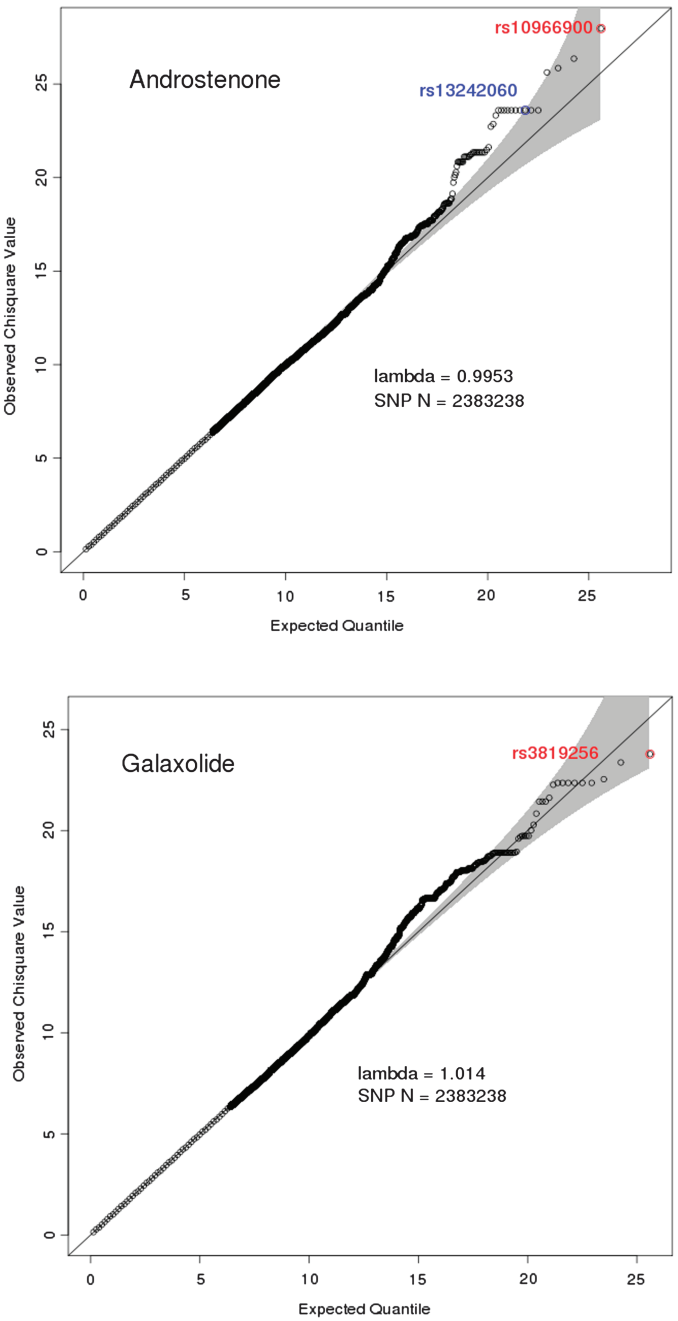


**Figure 4** Q–Q plots for androstenone (upper panel) and Galaxolide (lower panel). The 95% confidence interval is shown in gray. The stairstep feature of the plots reflects the 0–5 rating of perceived intensity. Both lambda values are near 1.0.

that act in a general way to reduce gene expression for all olfactory receptors, or subsets of receptors, for example, transcription factors or enhancer elements distant to an olfactory receptor gene or cluster of receptor genes.

### Methodological considerations for measures of olfaction

When measuring olfaction, there is a trade-off between the amount of time that can be devoted to testing and the

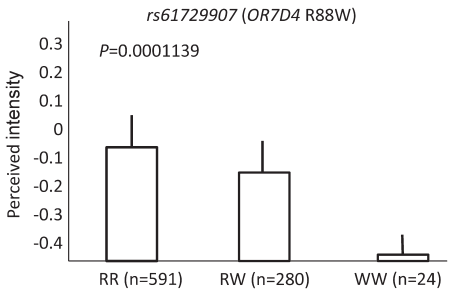


**Figure 5** Association between sensitivity to the odor of androstenone and *rs61729907* (*OR7D4* R88W) in the discovery sample. This marker was typed separately and was not in the original marker set for the genome-wide association panel. *P* value is from one-way analysis variance. Error bars denote SD.

accuracy of the tests employed. We used a scratch-and-sniff method for the genome-wide study, which is suitable for screening large numbers of people, and although crude, it was effective: rates of general anosmia agreed with previous reports (Gilbert and Kemp 1996; Feldmesser et al. 2007; Triller et al. 2008), and we were able to replicate a previously reported genotype–phenotype result using phenotype data collected with this method. We also used a second phenotyping method for the replication sample, which involved subjects smelling and rating single concentrations of odorant for intensity. Results from this method also worked well: the phenotype was similar to that of other methods, and the genotype association was also detected, with fewer subjects than with the scratch-and-sniff technique. However, we acknowledge that the heterogeneity of methods between the discovery and the replication samples is a limitation of this study.

## Conclusions

Heritability of perceived intensity for the musky odor Galaxolide was similar to that of androstenone, an odorant for which a genetic influence has been previously established. Results from genome-wide association analyses suggest that several genetic factors with small individual effects underlie heritability of androstenone and Galaxolide perception. We found an association between androstenone sensitivity and a genomic region without olfactory receptors, and we also found that a previous association with an olfactory receptor was difficult to replicate unless additional targeted genotyping was undertaken.

## Electronic and Chemical Resources

### Websites

http://www.ncbi.nlm.nih.gov/projects/SNP/, http://hapmap.ncbi.nlm.nih.gov/, http://www.ensembl.org/index.html.

### CAS and IUPAC

Androstenone CAS: 8339-16-7, I10,13-Dimethyl-1,2,4,5,6,7,8,9,11,12,14,15-dodecahydrocyclopenta[a]phenanthren-3-one; Galaxolide CAS: 1222-0505, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylindeno(5,6-c)pyran.

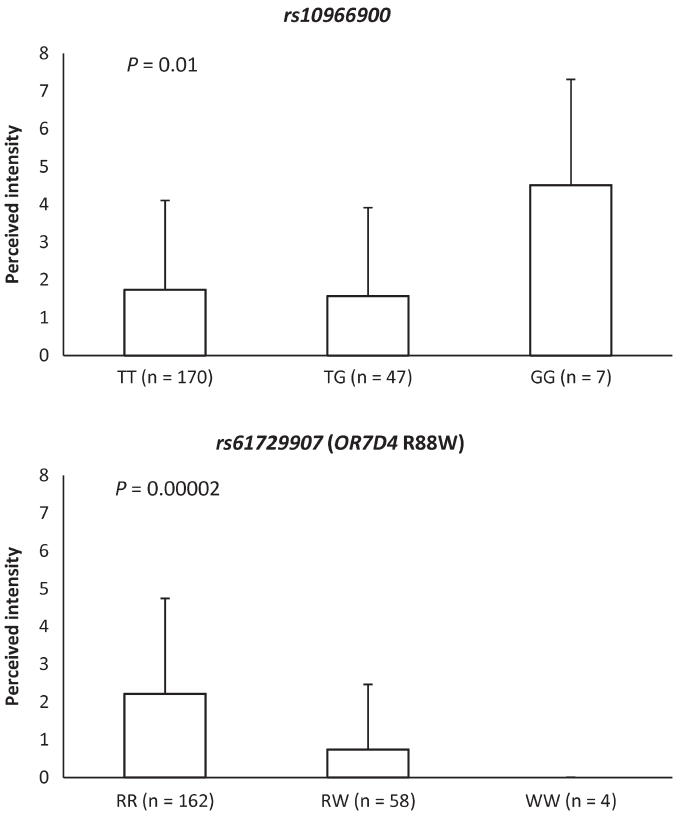


**Figure 6** Association between sensitivity to the odor of androstenone and alleles of SNP *rs10966900* (upper panel) and *rs61729907* (*OR7D4* R88W; lower panel) in the replication sample (*N* = 226). *P* values are from nonparametric Kruskal–Wallis one-way analysis of variance. Error bars denote SD.

## Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/

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